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INVESTIGATION OF FECAL CONTAMINATION OF FRESH PRODUCE IN FOUR TURKISH PROVINCES

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ABSTRACT

USAFE medical directors have long considered that the potentialities exist for intestinal-borne, infectious-disease transmission through consumption of raw produce grown in Turkey. A general understanding of sanitary conditions and habits of the people in this region justifies such an assumption. Only meager information concerning fecal contamination of fresh fruits and vegetables from the local economy has heretofore been gathered. The primary purposes of this study were to determine the possible sources and extent of produce contamination, and to evaluate the decontamination procedure recommended by TUSLOG directors of base medical services. In accomplishing these objectives, a laboratory procedure was developed for monitoring the efficacy of produce disinfection as practiced in TUSLOG food service facilities of Turkey.

This publication was prepared by CAPTAIN WILLIAM J. ROBERTSON, USAF, MSC, based on studies performed by personnel of the USAFE Epidemiological Flight (TUSLOG Detachment 36).

This publication has been reviewed and approved.

George R. Anderson

GEORGE R. ANDERSON
Lt Colonel, USAF, MC
Commander

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INVESTIGATION OF FECAL CONTAMINATION OF FRESH PRODUCE IN FOUR TURKISH PROVINCES

INTRODUCTION

In many areas of Europe and the Middle-Far Eastern Countries, locally-grown produce items are subject to contamination with organisms of fecal origin. Americans stationed in these areas are advised to wash and disinfect all local produce that is to be eaten without cooking. To establish or successfully monitor a program for prevention of intestinal-borne infections (e.g., typhoid, paratyphoid, diarrhea, dysentery, viral and parasitic diseases) disseminated via this medium, the nature and extent of fecal contamination of produce items in a given area must be known. In countries of the above regions, hazardous contamination of fresh fruits and vegetables may result from any one or more of the following:

1. Fertilization of crops with by-products of human or animal wastes. Of the two, the former is obviously more significant from the human-health point of view.

2. Irrigation of crops with fecal or sewage-polluted water.

3. Insanitary practices in handling produce during harvesting and marketing.

Although crops grown on or in the ground are more likely to sustain contamination from soil containing fecal organisms, aerial grown fruit commodities may also come in contact with soil during harvest seasons. On the other hand, produce items may be grown in the absence of fecal organisms only to be doused with polluted water or handled by individuals with fecal-contaminated hands. There is even some insecurity in ingesting nondecontaminated citrous fruits from certain areas, since it is impossible to peel the items without transferring fecal organisms to the interior pulp.

In 1957, personnel of TUSLOG Detachment 36 reported that local market produce in Izmir and Adana, Turkey, was found to be contaminated with organisms of fecal origin. [1] It was recommended that all produce be carefully washed and chemically decontaminated before consumption without cooking. For the past several years, the USAF Medical Service in Turkey has stressed that fruits and

vegetables of the above category be handled as outlined in Health Bulletins. [2] The decontamination procedure commonly recommended is briefly described stepwise below:

1. Avoid contamination of kitchen work areas by keeping fresh produce on paper or any suitable protective covering which can be readily discarded.

2. Remove all visible dirt and foreign matter from the produce by washing in mild detergent water and rinsing thoroughly.

3. Remove inedible portions such as roots, stems, and spoiled leaves, and again rinse the items thoroughly.

4. Soak produce items for 30 minutes in a solution containing 1 tablespoon of Clorox per gallon of water.

Visual observation and a general understanding of the sanitary conditions in Turkey today strongly suggest that raw produce consumption without disinfection to some degree would still be hazardous. The primary purposes of the currently reported investigation were: (1) to determine the degree of contamination of market and field crops, (2) to gain information concerning contamination sources, (3) to evaluate the above decontamination procedure, and (4) to develop a reliable laboratory method for analyzing fresh produce after Clorox decontamination. The latter procedure would be useful in monitoring produce decontamination at food service facilities.

Fresh produce items commonly eaten raw were purchased from local vendors of Izmir and examined for total coliform and enterococcus (fecal streptococcus) count before and after treatment using the decontamination procedure described. Demonstration of Escherichia coli and/or enterococci on a given produce item was considered to be evidence of fecal contamination. More recent investigations have given strong support to use of the latter organisms as fecal indicators. [3,4,5] Coliform-Enterococcus analyses were also performed on fresh produce samples collected directly from fields of a number of farms located in the provinces of Izmir, Antalya, Konya, and Kayseri. These studies were accomplished to determine the extent of field versus market produce contamination.

The Izmir markets were observed for sanitary conditions and practices related to contamination of fresh fruits and vegetables. While collecting farm samples, the kinds of fertilizer used, water sources for irrigation, and procedures employed in harvesting and shipment to market were established by observation and inquiry. The investigations were conducted over a fifteen-month period, beginning in February 1962, which allowed for seasonal variations in crop production.

MATERIALS AND METHODS

Isolation of Coliform and Fecal Streptococci

During the period, produce samples were obtained from fifteen to twenty different market sources located in five sections of Izmir. This included markets frequented by Americans. Not more than 6 samples were taken during any given week, and oftentimes a month or two would lapse when no samplings were made. Consequently, contamination monitoring was not restricted to any given source or period of time.

Each type of produce item was collected in separate, sterile paper bags at the various sources. Only the market attendants were allowed to handle and place fresh fruits and vegetables in the sterile bags. The samples were promptly returned to the laboratory and processed immediately, or refrigerated until analyses were performed within 18 hours. By employing flame-sterilized tongs and sterile paper, laboratory contamination of produce samples or cross-contamination of one sample to another was avoided. A procedure was devised for suspending known quantities of produce in buffer solution and arriving at the approximate number of coliform and enterococcus organisms per gram by culturing aliquots of the buffer. The laboratory procedure was also such to emulate produce decontamination as recommended by the directors of base medical services in Turkey. However, for the sake of maintaining proper bacteriological control, the hands of the technician were not allowed to touch the items at any time, which is not the case with produce decontamination in the home or in food service facilities. When received, the market items exhibited evidence of having been rinsed to remove most of the visible dirt and debris. Before analysis, it was necessary to

remove spoiled leaves and trim off root structures with a sterile knife.

Previous laboratory studies had revealed that 11 grams of produce are approximately equal to 1 gram of produce with most of the water removed. This was determined by grinding 11-gram quantities of various kinds of produce to pulp in a mortar and draining off the water before re-weighing. Therefore, 11 grams of unground produce placed in 99 mls of sterile buffer solution approximate a 1:100 weight to volume dilution. With this in mind, the produce samples were examined as follows:

1. Each produce sample was aseptically weighed using flame-sterilized tongs and sterile weighing paper and placed in individual sterile, 6-liter museum jars with paper covers.

2. Sufficient sterile milk dilution (phosphate) buffer [6] was added to each jar to yield the desired 1:100 dilution. For example, 150 grams of tomatoes required 1,350 mls of buffer because---

$$11 \text{ grams} : 99 \text{ mls} = 150 \text{ grams} : X$$
$$11X = \frac{14,850}{11} = 1,350 \text{ mls}$$

It was necessary to analyze fewer numbers of heavier and/or bulkier items to secure the proper ratio in the 6-liter capacity jars. As a general rule, individual examination lots consisted of 1 lettuce head, 3 large tomatoes, 20-30 radishes, 6-8 onions or leeks, 4-6 bell peppers, 1-3 oranges, etc., depending upon the size and weight of a single produce item.

3. Contaminating bacteria were dislodged from suspended samples by rotating the jars for 5 minutes. During this period, flame-sterilized tongs were used to turn and repeatedly submerge large produce items to facilitate thorough washing.

4. For coliform culture, 1.0 and 0.1-ml volumes of dilution buffer from each suspension were pour-plated using sterile, melted MacConkey's Agar (Difco Laboratories) at 45 C.

5. Enterococcus isolations were accomplished by inoculating 0.1, 1.0, and 10-ml quantities each of buffer to an individual series of 3 tubes containing Azide Dextrose Broth. Culture tubes

receiving 0.1 and 1.0-ml portions contained 10 mls of single-strength broth. Ten-ml buffer portions were inoculated to culture tubes containing 10 mls of double-strength Azide Dextrose Broth (see outline below). This procedure for presumptive determination of the Most Probable Number (MPN) of enterococci is described in Standard Methods.[7]

Azide Dextrose Broth	Buffer Samples Inoculated Each Tube
3 10-ml tubes single-strength	0.1 ml
3 10-ml tubes single-strength	1.0 ml
3 10-ml tubes double-strength	10.0 mls

6. Liquid household detergent in an amount equivalent to 1.0 ml per 1,000 mls of dilution buffer was then added to each produce suspension. The suspensions were swirled 1-2 minutes to wash the items.

7. Detergent-buffer solutions were aseptically decanted from the jars through a flame-sterilized wire screen cover. This was an old, metal-framed, laboratory animal jar cover composed of heavy-meshed wire with three triangular-spaced metal knobs on top. The cover was a close fit for the jar tops and was easy to handle during flame-sterilization and decanting of fluid from the produce samples.

8. All samples were rinsed at least three times in tap water, again employing the sterile wire cover to prevent laboratory contamination.

9. Following the third rinsing, samples were resuspended in Clorox solution equivalent to 1 tablespoon (15 mls) per gallon of tap water. Larger items such as lettuce heads naturally required larger volumes of disinfectant to allow submersion.

10. Leafy items of produce were intermittently turned and re-submerged with sterile tongs to insure contact of the Clorox water with all parts of the plant.

11. After 30 minutes of soaking, the Clorox water was drained from the samples as thoroughly as possible.

12. Sterile milk dilution buffer in an amount equal to one-half the original volume required to obtain a 1:100 dilution (step 2) was then added to each produce sample.

13. Following thorough washing of items by rotation of the jars, cultures for coliform and fecal Streptococcus count were prepared as previously described (steps 4 and 5).

14. The following controls were prepared for the above procedure:

a. Media-Glassware Control - A plate containing only MacConkey's Agar was poured from each flask of medium used in the previous pour-plating of produce wash-samples. Uninoculated tubes of each batch of Azide Dextrose Broth were also incubated.

b. Buffer Control - A 1-ml sample of each lot of sterile buffer used was pour-plated using MacConkey's Agar; 0.1, 1.0, and 10-ml amounts tubed in appropriate quantities of Azide Dextrose Broth.

c. Tap Water Control - The tap water used in rinsing samples on any given day's run was examined in the same manner as the buffer.

15. All pour-plate cultures were incubated for 48 hours at 35 C before examination. The Azide Dextrose Broth cultures which yielded growth at 24 hours at 35 C were subcultured as described below. Non-turbid broth cultures were incubated for another 24-hour period.

Determination of Coliform Count

At the end of the incubation period, coliform (pink-red) colonies of each MacConkey's Agar pour-plate were counted using a Quebec Colony Counter. The numbers of coliform colonies counted for 1.0 and 0.1-ml platings of buffer originally used in suspending produce samples (step 4) were multiplied by 100 and 1,000, respectively, to obtain the number of coliform organisms per gram of sample. Pour-plate counts of 1.0 and 0.1-ml quantities of buffer added to produce samples after Clorox decontamination (step 12) were multiplied by 50 and 500, respectively, in computing coliforms per gram.

Examination for Escherichia coli

A primary MacConkey's Agar pour-plate culture from each produce sample was examined as follows:

1. Using a straight inoculating needle, growth from coliform appearing colonies was carefully picked to individual Triple Sugar Iron (TSI) Agar slants. At least 3 separate colonies were examined in this manner.

2. After incubating the TSI Agar slants for 18-24 hours, tubes exhibiting typical acid and gas formation of coliform organisms were inoculated to Indole, Methyl red, Voges-Proskauer, and Simmon's Citrate (IMViC) media. [7]

3. Following appropriate incubation (usually 24-48 hours) of IMViC media, the coliform species were identified by comparing reactions with the chart shown below:

Organism		Indole	Methyl Red	VP	Citrate
Escherichia coli		+	+	-	-
	or				
		-	+	-	-
Citrobacter freundi*		-	+	-	+-
(Escherichia)	or				
		+	+	-	+
Klebsiella-Aerobacter		-	-	+	+-
	or				
		+-	-	+	+

*Produces H_2S in TSI Agar

Determination of Enterococcus Count

Azide Dextrose Broth tubes inoculated with aliquots of buffer before and after Clorox decontamination were examined at 48 hours for growth. The presence or absence of turbidity was recorded for each series of three broth tubes representing 0.1, 1.0, or 10-ml quantities of buffer. Positive growth tubes were individually subcultured to tubes of Tripticase Soy Broth containing 6.5 percent NaCL.

Tubes of this medium yielding no growth after 48 hours' incubation were discarded as negative for fecal streptococci, while turbid cultures were inoculated to blood agar plates. The latter were incubated for 24 hours under increased carbon dioxide at 35 C. Any fecal streptococci present were identified on the basis of colony appearance, Gram-staining, and catalase testing. These procedures satisfy the requirements for classification of enterococci outlined in Bergey's Manual. [8]

MPN of fecal streptococci for any given produce sample was computed by comparing the number of confirmed positive Azide Dextrose Broth tubes with MPN Table 31, page 506 of Standard Methods [7] for buffer aliquots examined. The MPN values determined represented enterococci per gram of produce rather than per 100 mls as shown in the Table, since original produce suspensions approximated proportions of 1 gram in 100 mls of buffer. Furthermore, MPN counts read from the Table on specimens analyzed after Clorox decontamination were doubled, since only one-half volumes of buffer were added back in step 12.

Examination of Lettuce Directly from the Field

Lettuce heads were collected directly from fields of four farms in a major produce growing area near Izmir. Using aseptic precautions, laboratory technicians cut the plants loose from their root structures and placed each head in a separate sterile bag. In the laboratory, visible dirt and debris were rinsed from each individual plant with tap water, since this was the minimum treatment given produce items prior to their being sold in the market. The lettuce heads were then examined for coliform and enterococcus organisms to determine the extent of fecal contamination of field crops. The laboratory procedures employed were the same as those previously described for non-Clorox'd produce items.

It was necessary to modify produce analysis procedures in examining samples collected during the overland field surveys of Antalya, Konya, and Kayseri. For these studies, 1-gram samples of each produce item were aseptically suspended in 99 mls of sterile phosphate buffer contained in wide-mouthed, screw-capped jars. After vigorous shaking, each suspension was processed for coliform organisms as shown earlier. For enterococcus count, 0.1 and 1.0 ml of buffer from each suspension

were passed through millipore filters, which were subsequently cultured on M-Enterococcus Agar. This procedure is the same as that described for water sample analysis in Standard Methods for Examination of Water and Wastewater. [7]

RESULTS AND DISCUSSION

In Table I, the coliform-enterococcus isolations from produce samples collected at Izmir markets are shown. Coliform and/or enterococcus organisms were recovered from all samples before washing and Clorox decontamination. Thirty-three of 34 samples examined yielded counts ranging from 300 to 300,000 or more coliform organisms per gram. Coliform counts of more than 10,000 per gram were demonstrated on 18 (53 percent) of the total samples examined. Although only 6 of the coliform positive specimens (18.2 percent) were found to include Escherichia coli, the overall coliform counts largely represent fecal contamination, if enterococci are to be regarded as reliable indicators. Twenty-eight (approximately 85 percent) of the coliform positive samples also contained enterococcus counts ranging from 3.6 to 1,100 organisms per gram.

The detergent washing-Clorox decontamination procedure was quite effective in diminishing coliform-enterococcus counts on samples tested. More than two-thirds of the samples, including certain items with initial coliform counts above 300,000 per gram, were reduced to zero coliforms. Items previously contaminated with enterococci were rendered free of the organisms in all but four instances. Ten coliform positive produce samples were not completely decontaminated. Counts of these items were decreased from a range of 5,800 to 300,000 plus coliforms per gram to 25 to 5,000 per gram. In all probability the organisms survived as a result of their inclusion in clumps of soil or debris which were not removed during the detergent wash phase. Under such conditions the cells could well have been inaccessible to the Clorox solution. Subsequent dispersion of the clumps in the process of determining post-Clorox counts would, in turn, render the organisms free for cultivation.

Analyses of 28 lettuce heads collected directly from the fields of four Izmir farms failed to yield enterococcus and

coliform organisms. The farmers stated that only well composted animal manure and/or commercial fertilizer was used on plots from which the samples were taken. It was later discovered, however, that fertilizer of human origin was used on farms immediately within the same district.

TABLE I

COLIFORM-ENTEROCOCCUS COUNTS PER GRAM OF IZMIR MARKET PRODUCE - BEFORE AND AFTER WASHING AND CLOROX TREATMENT

Produce Samples 15-20 Different Sources	Before Treatment (Plate Count) Coliforms	Before Treatment (MPN) Enterococci	After Treatment (Plate Count) Coliforms	After Treatment (MPN) Enterococci
Tomatoes				
Sample 1	9,925*	39	25	0
Sample 2	30,000*	19	200	6
Sample 3	400	0	0	0
Sample 4	5,800	460	25	0
Lettuce				
Sample 1	94,000	1,100	0	0
Sample 2	8,000	160	25	0
Sample 3	5,800	43	0	0
Sample 4	12,000	9.1	0	0
Sample 5	6,300	39	0	3
Sample 6	6,400	460	0	0
Sample 7	43,000	120	0	0
Sample 8	41,000	290	0	0
Sample 9	60,000	34	0	0
Sample 10	246,000	>460	0	0
Leeks				
Sample 1	>300,000	28	0	0
Sample 2	21,000*	3.6	0	0
Sample 3	>300,000	3.6	5,000	0
Sample 4	85,500	9.1	2,025	0

*Counts from which Escherichia coli organisms were isolated.

TABLE I (Continued)

Produce Samples 15-20 Different Sources	Before Treatment (Plate Count) Coliforms	Before Treatment (MPN) Enterococci	After Treatment (Plate Count) Coliforms	After Treatment (MPN) Enterococci
Onions				
Sample 1	>300,000*	>460	0	0
Sample 2	>300,000	3.6	0	0
Sample 3	>300,000	15	1,600	0
Sample 4	197,000	15	0	0
Radishes				
Sample 1	9,250	240	100	0
Sample 2	1,900	11	0	0
Sample 3	7,800	21	0	23
Sample 4	58,000	20	1,100	0
Oranges				
Sample 1	600	0	0	0
Sample 2	600	0	0	0
Sample 3	0	9.1	0	0
Tangerines	700	0	0	0
Cherries	300	0	0	0
Plums	2,100	0	0	0
Grapes	600*	460	0	29
Carrots	20,000*	100	300	0

*Counts from which Escherichia coli organisms were isolated.

In Tables II, III, and IV, the results of produce analyses are summarized for samples collected directly from fields of farms within Antalya, Konya, and Kayseri Provinces. As indicated, human as well as animal wastes were being used for fertilizer in many

TABLE II

COLIFORM-ENTEROCOCCUS ANALYSES RESULTS
OF FIELD COLLECTED PRODUCE SAMPLES
ANTALYA PROVINCE

	Fertilizer Used (Human-Animal)	Coliforms/Gm	Enterococci/Gm
Farm No. 1			
1 sample (tomatoes)	A H	0	0
Farm No. 2			
1 sample (tomatoes)	A H	0	0
1 sample (tomatoes)	A H	0	6,500
Farm No. 3			
1 sample (tomatoes)	A H	0	0
1 sample (tomatoes)	A	0	0
Farm No. 4			
1 sample (tomatoes)	A	0	0
Farm No. 5			
2 samples (tomatoes)	A	0	0
Farm No. 6			
3 samples (tomatoes)	A	0	0
Farm No. 7			
1 sample (peppers)	None	0	0
1 sample (tomatoes)	None	0	0
1 sample (tomatoes)	A	0	0
Farm No. 8			
1 sample (tomatoes)	A	0	0
Farm No. 9			
1 sample (tomatoes)	None	3,700*	0
1 sample (tomatoes)	A H	30,000*	0
1 sample (tomatoes)	None	0	0

*Including E. coli

KEY: A - animal waste fertilizer H - human waste fertilizer

areas from which samples were taken. Municipal garbage was also being utilized as fertilizer to some extent in Konya and Kayseri. On the basis of coliform and/or enterococcus isolations, 12 (29 percent) of 41 samples collected from the Antalya-Konya-Kayseri farms were contaminated. Three were positive for coliforms only (including E. coli); 1 contained coliforms and enterococci; and 8 yielded enterococci exclusively. Coliform counts ranged from 3,700 to 30,000 per gram, enterococcus counts from 100 to 9,000 per gram. It was interesting to note that the greatest number of

TABLE III

COLIFORM-ENTEROCOCCUS ANALYSES RESULTS
OF FIELD COLLECTED PRODUCE SAMPLES
KONYA PROVINCE

	Fertilizer Used (Human-Animal-Garbage)	Coliforms/Gm	Enterococci/Gm
Farm No. 1			
1 sample (lettuce)	A	0	2,000
1 sample (onion)	A	0	0
Farm No. 2			
1 sample (lettuce)	A	0	0
Farm No. 3			
1 sample (leek)	A G	0	0
1 sample (lettuce)	A G	0	0
Farm No. 4			
1 sample (lettuce)	A H	0	0
1 sample (lettuce)	A H	4,000*	0
1 sample (lettuce)	A H	0	7,500
1 sample (lettuce)	A H	0	100
1 sample (lettuce)	A H	0	8,000

*Including E. coli

KEY: A - animal waste fertilizer H - human waste fertilizer
G - garbage fertilizer

contaminated samples came from fields where human fecal material was being incorporated for fertilizer. Ten of 22 samples from such areas were positive for fecal bacteria and were representative of six different farms. On the other hand, only 2 of 19 samples from eleven farms not using human excrement for fertilizer were positive for fecal organisms.

Insanitary conditions and practices in the harvesting and marketing of fresh produce were observed in Izmir. These findings were consistent with the laboratory results obtained on analysis of samples from the various markets and vendors. On the farms, harvested fruits and vegetables are often piled on the ground or placed in dirty woven baskets or bins. In some instances produce items are stored on dirty floors of barns or in small utility houses with adjacent privies until transported to market. Under these conditions flies, insects, and rodents are often attracted to the commodities. To prevent produce deterioration, "fresh-up" rinses are frequently applied using water from shallow wells and streams of questionable potability. The sorting out of produce items by farm workers with unclean hands appears to be a likely source of fecal contamination of produce for the following reasons. Soap is an expensive item, infrequently found in public latrines in Turkey; consequently, it is difficult for the laboring class and other individuals of low income to properly cleanse their hands after using the toilet. It is even difficult for them to become accustomed to using soap when it is offered. Furthermore, the common laborer does not generally use paper after defecation. Instead, he washes himself with water using his bare hand. The latter becomes so heavily contaminated that even scrubbing with soap and water may not remove all of the fecal organisms present. Evidence of the above practice is the small cans of water commonly observed in public latrines, as well as in latrines used by indigenous food handlers at TUSLOG installations. Detachment 36 personnel have frequently isolated Escherichia coli from the hands of indigenous workers employed at food service facilities.

Oftentimes produce items have been observed in transit via dirty open carts or in baskets on the sides of donkeys. On two occasions wagon drivers were observed dousing loads of fresh produce with water from sewage-laden streams. In the market, produce items are again handled directly by attendants in the process of setting up elaborate displays in boxes and baskets, often laden with dirt and foreign matter. Market attendants have been

frequently observed wetting down produce commodities with water from the Izmir municipal system. This constitutes still another source of fecal organism contamination, as surveys of many tap-water outlets throughout the city have revealed a 30-50 percent sample pollution rate. Refrigeration facilities for perishable produce items are usually lacking in the markets; consequently, spoilage is commonly observed and serves as an attractant for flies and other insects which enter through unscreened doors and windows. Rodents, particularly sewer rats, are known to inhabit many dwellings in Izmir and could well serve as a source of produce contamination.

TABLE IV

COLIFORM-ENTEROCOCCUS ANALYSES RESULTS
OF FIELD COLLECTED PRODUCE SAMPLES
KAYSERI PROVINCE

	Fertilizer Used (Human-Animal-Garbage)	Coliforms/Gm	Enterococci/Gm
Farm No. 1			
1 sample (beet tops)	A H G	4,350	9,000
1 sample (beet tops)	A H G	0	0
1 sample (parsley)	A H G	0	0
Farm No. 2			
3 samples (onions)	A H G	0	0
1 sample (onions)	A H G	0	300
Farm No. 3			
2 samples (lettuce)	A H G	0	0
Farm No. 4			
1 sample (lettuce)	A H	0	400
1 sample (parsley)	A H	0	100
1 sample (onions)	A	0	0
1 sample (lettuce)	A H	0	0

KEY: A - animal waste fertilizer H - human waste fertilizer
G - garbage fertilizer

Extensive interviews with farmers in the four provinces revealed that human wastes are being used for fertilizer on other farms, in addition to those from which field samples were taken. In urban areas, farmers are collecting from "septic" tanks and privies located within the cities. After transporting to the farm, the wastes are often promptly mixed with well-composted animal manure and, with little or no delay, washed into irrigation channels onto the fields. In other instances, such as that observed in Kayseri, municipal garbage and human wastes are mixed with animal manure and allowed to compost for considerable periods before washing onto the fields. In Izmir, the owner of a large farm consistently mixes human fecal material with lime and rice hulls prior to combining it with animal manure. The composite manure is allowed to ferment 15 days before mixing with water in a large, concrete bowl where clumps are broken into suspension with the bare feet. The manure is then washed out of the bowl into field irrigation trenches.

In the smaller towns and villages, it appeared that little or no attention is being given to aging of human feces before use. Wastes directly from private privies are simply mixed with soil or composted animal manure and washed onto the fields. In some villages and towns, sewage from the houses was observed draining directly into fields where items such as tomatoes, peppers, lettuce, cucumbers, and onions were being grown. Many village farmers expressed disappointment over human fecal material being in short supply. Tomatoes fertilized with human wastes were being grown in Antalya and marketed in Izmir, Ankara, Istanbul, Bursa, and Eskişehir.

CONCLUSIONS

The foregoing studies indicate that fresh produce items in Turkey undergo fecal organism contamination to the greatest degree during harvesting and marketing. This was evident upon comparing coliform-enterococcus counts of Izmir market produce with that obtained from analysis of produce samples collected directly from fields in Izmir, Antalya, Konya, and Kayseri Provinces. It was not considered necessary to analyze produce items directly from markets in cities of the latter three provinces, as sanitary conditions and practices during harvesting and marketing are essentially the same throughout Turkey.

Two major sources of market produce contamination appear to be direct handling of items with fecal-soiled hands and the repeated application of polluted water to prevent deterioration of the commodities. Another contributing factor is the exposure of produce items to insanitary environments during harvesting and transporting to market, as well as during storage in the shops. Examples of the latter are manifested by dirty storage containers and push-carts, lack of refrigeration to delay spoilage, and failure to prevent insects and rodents from gaining access to produce materials.

Fecal bacteria were isolated from a significant number of produce samples collected directly from fields where human wastes were being used for fertilizer. Conversely, samples from fields in which animal manure or fertilizer--other than human waste materials--were being employed yielded rare evidence of fecal contamination. Perhaps these results were influenced by the age of the fecal material used. Fresh animal and human wastes must be allowed to compost for a period of time, otherwise their use will be injurious to crops. During composting periods, fecal bacteria, including coliforms and enterococci, are largely destroyed. Aging of human feces before use is not essential provided the material is mixed with liberal amounts of well-composted animal manure or other fertilizer. This could well have been the case in areas from which contaminated produce samples were collected. In some instances contaminated samples were not full grown, and evidence of recent crop fertilization was noted in the fields. Under these conditions it is understandable that viable coliform and enterococcus organisms could be present. Furthermore, had samples been collected and examined at a later date, when certain produce items had reached full maturity, the contamination incidence might have been even less.

It can be stated, without reservation, that the opportunities for field contamination of produce from polluted irrigation water in the provinces visited are numerous. The great majority of water sources examined in agricultural areas by the Detachment 36 engineering section exhibited evidence of fecal pollution.

For reasons previously shown, fresh fruits and vegetables--regardless of their source in Turkey--should be thoroughly washed and decontaminated before consumption without cooking.

The recommended decontamination procedure evaluated in this study appears to be effective provided careful attention is given to each step. Initial pilot studies performed have revealed that neither the detergent wash phase nor Clorox disinfection stage can be safely omitted.

RECOMMENDATIONS

Preventive medicine personnel should frequently monitor produce washing and decontamination procedures as practiced in TUSLOG food service facilities within Turkey.

The following points should be stressed in supervising individuals whose duties are to disinfect fresh fruits and vegetables:

1. Soil and foreign material must be thoroughly removed from produce items prior to placing in Clorox solution. Excessively dirty items quickly dissipate chlorine to ineffective levels.

2. Leafy items such as cabbage and lettuce should be separated or peeled apart prior to placing in Clorox solution, thus allowing contamination deep within the folds to be reached by disinfectant.

3. Clorox solutions must be carefully measured to obtain the desired concentration equivalent of 1 tablespoon of Clorox per gallon of water. (75-100 ppm)

4. The required 30-minute period for soaking produce in Clorox solution must not be shortened.

5. Clorox solutions should not be overloaded, otherwise effectiveness of disinfection will be greatly reduced. Produce items should be freely suspended in one layer and weighted down to submersion with a wire screen or refrigerator shelving.

Samples from each shipment of liquid Clorox and powdered chlorine concentrate(Stock No. 6840-270-8172) received through commissary stores should be examined by preventive medicine personnel before sale or issue. Representative samples should yield the desired concentration, 75-100 ppm, upon adding 1 tablespoon of Clorox solution to a gallon of water, or as directed on the package for chlorine powder.

As a part of the required incoming medical orientation of all military personnel and dependents assigned to Turkey, the information contained in this study should be utilized as a basic reference.

The following laboratory procedure may also be used for periodic evaluation of produce decontamination in food service facilities. The maximum allowable coliform count for chlorine decontaminated produce was derived from the studies of Izmir market produce cited earlier in this report

COLIFORM COUNT DETERMINATION OF CLOROX DECONTAMINATED PRODUCE

MATERIALS

1. Sterile Paper Bags

2. Sterile 8x10-1/2 inch Paper - for weighing produce samples

3. Harvard Trip Balance

4. Sterile Buffer Blanks - wide-mouthed, screw-capped jars containing 99 mls or 198 mls (see paragraph 4a of Laboratory Procedures) of buffer prepared as follows:

   a. Stock Buffer Concentrate - Dissolve 34 grams of potassium dihydrogen phosphate (KH_2PO_4) in 500 mls of distilled water, adjust pH to 7.2 with 1 N NaOH solution, and make up to 1 liter with distilled water. Sterilize at 15 lbs pressure for 15 minutes and store in refrigerator to prevent bacterial growth. This stock solution must be kept sterile.

   b. Working Buffer Solution - Dilute 1.25 mls of the stock concentrate to 1 liter with distilled water. Use this solution for analysis of produce items.

5. Tongs - for aseptic handling of produce items

6. Knife with a 4-6 inch blade - for cutting produce items in the process of weighing samples

7. Alcohol and Gas Burner - for flame sterilization of tongs and knife blade

8. Sterile Petri Dishes - standard size, plastic or glass

9. MacConkey's Agar

COLLECTION OF SAMPLES

Aseptically collect fresh salads in separate, sterile paper bags from the serving lines of food service facilities. If desired, individual produce items which have been washed and treated with Clorox solution may be aseptically collected from the kitchen. Allow only the food handler responsible for preparing fresh salads to place each sample in the sterile bag. Salads containing excessive water or liquid dressings should be carefully drained prior to placing in collection bags. It may be necessary to collect moisture laden items in sterile, wide-mouthed jars with screw-caps to avoid contamination.

LABORATORY PROCEDURES

1. All produce samples should be analyzed as soon as possible after collection.

2. Extreme care should be taken to prevent laboratory contamination of specimens or cross-contamination of one specimen to another.

3. The controls listed at the end of this procedure should always be run in parallel with sample analysis.

4. Previous laboratory studies have revealed that 11 grams of produce are approximately equal to 1 gram of solid material. This was established by reducing 11-gram quantities of various items to pulp in a mortar and draining off the water before re-weighing. Therefore, 11 grams of produce placed in 99 mls of sterile buffer solution approximates a 1:100 dilution. With this in mind, examine each produce item as follows:

    a. Weigh 11 grams of samples aseptically (using flame-sterilized tongs and sterile weighing paper) and place in a jar containing 99 mls of sterile buffer. If examination of

a larger quantity is desired, suspend 22 grams of sample in 198 mls of sterile buffer.

b. Shake the suspended sample for 5 minutes to dislodge contaminating bacteria.

c. Remove 1.0 and 0.1-ml volumes of dilution water from the produce suspension and place in separate, sterile Petri dishes.

d. Add sterile, melted MacConkey's Agar at 45 C to the Petri dishes and mix their contents by gently rotating each dish on the table top.

e. Upon solidification of the agar, invert the dishes and place them in a 35-37 C incubator for 48 hours.

f. Count the coliform (pink-red) colonies on each plate. Multiply the number of colonies counted for the 1-ml sample by 100, the 0.1-ml sample by 1,000, to obtain number of coliforms per gram of produce sample.

COLIFORM IDENTIFICATION

Confirming the presence or absence of Escherichia coli is useful when coliform counts are in excess of 5,000 per gram of produce sample. Isolation of E. coli from the MacConkey's Agar pour-plates provides evidence that fecal contamination of the produce has occurred. This is accomplished as follows:

a. Using a straight inoculating needle, carefully pick growth from coliform appearing colonies to individual Triple Sugar Iron (TSI) Agar slants. At least 3 separate colonies from a given sample should be examined in this manner.

b. After incubating the TSI Agar slants for 18-24 hours, sub-culture tubes exhibiting typical acid and gas formation of coliform organisms to Indole, Methyl red, Voges-Proskauer, and Simmon's Citrate (IMViC) media.

c. Following appropriate incubation (usually 24-48 hours) of IMViC media, identify the coliform species by comparing reactions with the following chart:

Organism		Indole	Methyl Red	VP	Citrate
Escherichia coli		+	+	-	-
	or				
		-	+	-	-
Citrobacter freundi* (Escherichia)		-	+	-	+-
	or				
		+	+	-	+
Klebsiella-Aerobacter		-	-	+	+-
	or				
		+-	-	+	+

*Produces H_2S in TSI Agar

INTERPRETATION

1. Coliform counts greater than 5,000 per gram of produce represent fairly heavy contamination of possible fecal origin. Produce from a given facility should definitely be considered potentially hazardous when such counts are frequently obtained in conjunction with occasional Escherichia coli isolation.

2. Counts of more than 5,000 coliforms per gram with or without E. coli demonstration are conclusive evidence of improper decontamination and/or handling of salad items in food service establishments.

LABORATORY CONTROLS

1. Media-Glassware Control - A plate containing only MacConkey's Agar should be poured and incubated to serve as a sterility check on each lot of glassware and media used in the analyses.

2. Buffer Control - A 1-ml sample from one of the buffer blanks sterilized along with others used should be pour-plated with MacConkey's Agar.

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